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07/695,1991 03/02/91 RECORDED

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EXAMINER

STACEY R. SIAG, PH.D.  
HOFFMAN-LA ROCHE INC.  
340 KINGSLAND STREET  
NUTLEY, NJ 07110

PTXELIA, JR

ART UNIT PAPER NUMBER

15

DATE MAILED:

3/12/92

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

This application has been examined  Responsive to communication filed on 9/9/92  This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), — days from the date of this letter.  
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1.  Notice of References Cited by Examiner, PTO-892. 2.  Notice re Patent Drawing, PTO-948.  
3.  Notice of Art Cited by Applicant, PTO-1449. 4.  Notice of Informal Patent Application, Form PTO-152.  
5.  Information on How to Effect Drawing Changes, PTO-1474. 6.

Part II SUMMARY OF ACTION

1.  Claims 1-22 are pending in the application.

Of the above, claims \_\_\_\_\_ are withdrawn from consideration.

2.  Claims \_\_\_\_\_ have been cancelled.

3.  Claims \_\_\_\_\_ are allowed.

4.  Claims 1-22 are rejected.

5.  Claims \_\_\_\_\_ are objected to.

6.  Claims \_\_\_\_\_ are subject to restriction or election requirement.

7.  This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8.  Formal drawings are required in response to this Office action.

9.  The corrected or substitute drawings have been received on \_\_\_\_\_. Under 37 C.F.R. 1.84 these drawings are  acceptable.  not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10.  The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_ has (have) been  approved by the examiner.  disapproved by the examiner (see explanation).

11.  The proposed drawing correction, filed on \_\_\_\_\_, has been  approved.  disapproved (see explanation).

12.  Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has  been received  not been received  been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_.

13.  Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14.  Other

EXAMINER'S ACTION

Art Unit 1814

Applicants' arguments filed on 9/9/92 paper No. 12, have been fully considered and are deemed to be persuasive to overcome most of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

The disclosure is objected to because of the following informalities: the wording of Claim 11 is grammatically awkward in the phrase "A method ..., said method comprises...". Substitution with either "wherein said method comprises..." or "said method comprising..." is suggested.

The following is a quotation of 35 U.S.C. § 103 which forms the basis for all obviousness rejections set forth in this Office action:

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Claims 1-22 are rejected under 35 U.S.C. § 103 as being unpatentable over Sutherland et al. in view of Mullis et al. (Reference AD, U.S. Patent No. 4,683,195).

Art Unit 1814

Sutherland et al. disclose methods for the use of fluorescent dyes including in particular ethidium bromide for measurement of polymerization of nucleic acids during PCR amplification. They disclose that these dyes can be provided directly in the PCR reaction and that the dyes have a greater fluorescence when bound to double stranded DNA than when either bound to single stranded DNA or unbound.

Mullis et al. disclose that amplification of nucleic acids by PCR allows the detection of very rare nucleic acids present in a large excess of other nucleic acids and that detection of amplified nucleic acids is useful for the detection of genetic and infectious disease. They further disclose that PCR selectively synthesizes only the selected target DNA during amplification.

Therefore, it would have been obvious to one of ordinary skill in the art to use the method of detecting polymerization by use of fluorescent dyes during a PCR amplification to detect the target nucleic acid of the amplification reaction because this method is very simple and minimizes sampling and handling errors (see Sutherland et al. column 16, lines 64-69).

In regards to Claim 10, the ordinary skilled artisan would have known that one could quantitate the amount target DNA originally present in the amplification reaction from the measured change in fluorescence by a simple comparison to a

Art Unit 1814

standard curve of the amount of fluorescence change produced by a given amount of initial DNA.

In regards to Claims 12-16, the use of optic fibers to continuously monitor the fluorescence of a solution is old and well known in the art and as such it would have been obvious to use one in order to monitor the synthesis of the target nucleic acids during the amplification reaction for the added simplicity of not having to remove aliquots of the reaction at various times.

In regards to Claims 17-22, as Sutherland et al. disclose that the fluorescent dye can be provided directly within the starting PCR buffer, it would have been obvious to include the dye within the buffer of a kit for detecting amplified target nucleic acids, such as the one disclosed by Mullis et al. for the added convenience of minimizing the amount of time needed to prepare the reaction.

Applicant's arguments filed 9/9/92 have been fully considered but they are not deemed to be persuasive.

Applicant argues that Examiner has misread the Sutherland et al. reference. This is in fact not the case, as in column 16, lines 58-69 Sutherland et al. clearly state "... the fluorescent dye was mixed with the template and other polymerization reagents prior to contact with the DNA polymerase to be detected. The polymerase chain reaction was then initiated by the addition of

Art Unit 1814

aliquots of DNA polymerase and fluorescent signals measured continuously over time." (emphasis added) and in column 17 lines 26-30 they state "reaction was initiated by adding a solution of DNA polymerase (isolated from Thermus aquaticus as identified above in Example 1) at various concentrations." Therefore, Sutherland et al. clearly do teach the use of fluorescent dyes within a polymerase chain reaction as stated by the examiner. Although Sutherland et al. do not use ethidium bromide in Example 6, they state in column 3, lines 52-59 "The advantages of this invention are achieved by using certain dyes which exhibit a measurable difference in signal when bound to a double stranded DNA molecule as opposed to when they are bound to the corresponding single stranded template. We have found a number of dyes which exhibit such changes. These changes can be used to monitor the rate of generation of double stranded DNA from the corresponding single stranded template" and in column 7, lines 55-66 and in Example 3, specifically mention ethidium bromide as being one of these useful fluorescent dyes.

Applicant also argues that the combination of references fails to suggest the introduction of intercalating agents into a PCR reaction mixture. This is clearly in error as Sutherland et al. explicitly teaches this as is discussed in detail above. Furthermore, Mullis et al. was included in the rejection only as evidence that the fluorescence produced would result from only

Art Unit 1814

the target DNA as during PCR only target DNA is produced even if the original solution contains a large amount of non-target DNAs.

Applicant argues that the prior art contains references teaching away from the invention by teaching that intercalating agents are inhibitory of DNA synthesis. This is not convincing because Sutherland et al. clearly teaches that fluorescent dyes can be used within polymerase chain reactions. Furthermore, Sutherland et al. was published considerably after the prior art references referred to by applicant showing that the teachings therein are incorrect at least in respect to the levels of dye (i.e. 1-5  $\mu$ M in general and 1.75  $\mu$ M specifically in Example 6) taught by Sutherland.

Applicant also argues that he has shown surprising results in showing that intercalating agents need not inhibit DNA polymerase activity. This is not convincing because this is not in fact surprising in view of the teachings of Sutherland et al. as discussed in detail above.

**THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE

Serial No. 07/695, 201

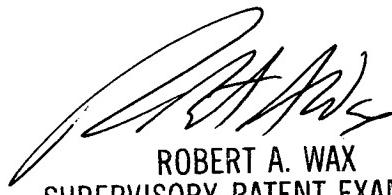
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Art Unit 1814

MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Rebecca Prouty whose telephone number is (703) 308-4000.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.



ROBERT A. WAX  
SUPERVISORY PATENT EXAMINER  
GROUP 180